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USING DIFFERENT GRAIN SUBSTRATES FOR SPAWN PRODUCTION AND MAXIMIZATION OF OYSTER MUSHROOM (*Pleurotus florida*) PRODUCTION

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ABSTRACT: Optimizing spawn quality is crucial for successful mushroom cultivation, and it relies on grain formation. This study was carried out at Faculty of Agriculture, Zagazig University, Egypt during the consecutive seasons of 2021-2022. In this study, a hatchery was developed using various grains. The grain substrates, including maize, sorghum, wheat, and a mixture of maize: sorghum: wheat spawn (1:1:1), were inoculated with *Pleurotus florida* parent eggs. After incubation, the eggs were grown in rice straw substrate, exhibiting variable effects on the days from sowing to the first flushing (15-33 days). Notably, sorghum grain substrate demonstrated the highest productivity in yield quantity and fruit size, followed by the wheat-sorghum mixture in both seasons. The nutritional richness of sorghum and the 1:1:1 mixture played a pivotal role in stimulating robust fungal growth, influencing both yield quantity and morphological characteristics of the fruits. Considering these results and previous studies, the utilization of sorghum grain substrate is strongly recommended. Moreover, introducing Slim as a hatchery carrier can enhance hatching quality, fostering faster and healthier growth. This approach aims to maximize returns, ultimately leading to increased profits in mushroom cultivation.

Key words: Grain substrates, spawn, oyster mushroom.

INTRODUCTION

Oyster Mushroom (*Pleurotus ostreatus*), widely distributed in Egypt and often cultivated on rice straw **(Törős *et al.*, 2022)**. Oyster mushrooms are important for their high nutritional value, comprising vitamins, minerals, and natural phytochemicals, boasting high protein, fiber, and antioxidant levels, which enrich the immune system and overall health. Some studies suggest various mushroom species hold promise in combating illnesses such as cancer, allergies, diabetes, high cholesterol, and stress **(Effiong *et al.*, 2023)**. Additionally, they aid in promoting better sleep, relieving asthma symptoms, and potentially combating HIV **(Rahman *et al.*, 2022)**. Moreover, oyster mushrooms have shown efficacy in bioremediation and possess biologically active compounds with antimicrobial and antioxidant properties **(Waktola and Temesgen, 2020; Törős *et al.*, 2022)**. Oyster mushroom cultivation has garnered increased attention due to its ease of propagation, high nutritional quality, and ability to utilize various agricultural wastes including rice straw, cotton plants, wood, palm, *etc*. **(Seethapathy *et al.*, 2023)**.

Mushroom spawn, comprising mushroom mycelium on a substrate, serves as the planting material in cultivation. Four common types of spawn sawdust, grain, liquid, and stick are currently available with challenges in selecting suitable inoculum materials **(Jarial and Jarial, 2022; Ufitinema *et al.*, 2023)**. Factors like spawn grain substrates and environmental conditions including temperature, oxygen, carbon dioxide, humidity, light, and pH influence mycelial growth and fruit bodies production **(Karpagavalli *et al.*, 2024)**.

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Oyster mushroom *Pleurotus* spp. emerges as a potentially cost-effective and easily cultivable alternative **(Lucas *et al.*, 2023)**. The objective of this study is to investigate the impact of diverse spawning grain substrates on mycelial growth, faster spawn inoculation period, fruitbodies productivity and quality.

MATERIALS AND METHODS

This experiment was conducted at the Mushroom Research Laboratory (MRL), Department of Horticulture, Faculty of Agriculture, Zagazig University, Egypt. A lab experiment was conducted between 2021 and 2022 growth seasons.

Strain and Maintenance

The oyster mushroom strain (*Pleurotus florida*) used in this study obtained from the national climate laboratory at the National Research Center, Egypt. Cultivated on potato dextrose agar (PDA) slants at 25ºC for 7 days, it was stored at 4ºC and subjected to regular sub-culturing every four weeks.

Spawning Substrates

Four distinct grain crops served as mediums for mushroom spawn production including maize, sorghum, wheat grain spawn, and their mixture of maize: sorghum: wheat grain spawn (1:1:1).

Spawn Preparation

To prepare the grain spawn, grain substrates individually subjected to a 30-minutes boiling process, achieving a half-cooked state. Subsequently, these grains were dried using a central centrifuge and mixed with 2% calcium carbonate powder. The half-cooked grains were then carefully packed into 1 kg plastic bags, autoclaved, and sealed with cotton. Sterilization took place at 120°C for 20 minutes. The following day, spores sourced from pure cultures were used to inoculate the prepared grain spawn. The pure culture, a 15-18 days old spawn, was procured from the national climate laboratory at the Agriculture Research Center in Egypt. Incubation ensued at room temperature for 15 days.

Preparation of Rice Straw

Rice straw was chopped into particles (15-20 cm), soaking in tap water for 12 hours, draining excess water, and pasteurization in a life steam system at 80–90°C for 6 hours. The pasteurized rice straw was then left to attain room temperature **(Arisha, 2010)**.

Oyster Mushroom Cultivation

Rice straw substrates were removed and spread between rice straw layers within polyethylene bags (60 cm depth x 40 cm diameter). Each bag, containing 2 kg of dry substrate (approximately 6 kg wet), received spawn material distributed at a rate of 5% (w/w), equivalent to around 150 g per bag **(Arisha 2010)**.

Mycelial Growth

Polyethylene bags contained grains inoculated with spores were inoculated to an incubation room with a temperature of 24±3°C, reduced ventilation, and darkness, allowing full colonization for two weeks till full mycelium growth. Subsequently, the bags were transferred to the production room, maintaining a temperature of 16-20°C and a relative humidity of 80-90% using a foggy system. To facilitate fungal growth, polyethylene bags were perforated with holes measuring 1 cm in diameter, spaced 5 cm apart (50 holes per bag). This ensured sufficient aeration necessary for optimal mycelial development.

Data Recorded

Growth characters

During the first flush (first 15 days), two oyster mushroom clusters were selected from each treatment. Growth parameters and fruit bodies characteristic were recorded, including cap weight (g), cap diameter (cm), stipe weight (g), stipe diameter (cm), and stipe length (cm).

Yield and its components

Upon reaching the appropriate harvesting stage, all clusters were harvested, data were recorded. The recorded data included Number of clusters/bag, Number of fruit bodies/cluster, Average fruit body weight, Total fresh yield of mushroom/bag, Early yield/bag (calculated for the first 15 days from the beginning of harvesting), Average cluster weight, Early yield/ total yield ratio, and Total dry yield/bag.

Statistical Analysis

The obtained data were subjected to statistical analysis of variance according to **(Snedecor and Cochran 1980)** and means separation were done according to **(DB 1955)**.

RESULTS AND DISCUSSION

Oyster mushroom spawn, comprising mushroom mycelium on a substrate, serves as the planting material in cultivation. Four common types of spawn sawdust, grain, liquid, and stick are currently available with challenges in selecting suitable inoculum materials.

Yield and its Components

Fungal growth on different grain and its effect on yield and its components are shown in Table 1. Four different grain substrates were used as a substrate for spawning including sorghum, maize, wheat and a mixture of them (1:1:1). For total yield per bag (g) the three grain substrates maize, sorghum and the mixture gave high yield while wheat grain substrates showed lowest yield values in both seasons. Moreover, the sorghum grain substrate in both seasons significantly showed the highest total yield amount per bag.

For early yield per bag sorghum gain substrate showed the highest early yield per bag in both seasons, while wheat was the lowest. The mixture grain substrate early yield value came at the second rank in the first season. In the second season all of the treatments were significantly higher than wheat grain substrate. In addition, the number of fruit bodies per cluster gave the highest value when wheat grain substrates were used in the first season while maize substrate came in the first rank at the second season.

These results are in agree with **Jarial and Jarial (2022)** and **Sen *et al.* (2022)** who reported that sorghum grain substrate was the best for oyster mushroom production. In the current study wheat grain substrate showed lower performance than other grain substrates, on the contrary, many results disagree with these current results. The studies of **Jarial and Jarial (2022) and Verma *et al.* (2023)** showed that sorghum and wheat substrates showed almost similar performance and it was the best medium for spawning. This maybe that at the preparation of spawn the boing step of the grains should give half cooked grains to be suitable for inoculation. In the current study a fixed time was used for boiling all used grains, which resulted in overheated (well cooked) wheat grains there for it showed lower performance **(Ufitinema *et al.*, 2023)**.

Fruit Body Characteristics

It is clear from Table 2 that cap diameter was affected by using different grain substrates for spawning. In the first season maize grain substrate showed the highest cap diameter followed by wheat and sorghum then the mixed substrate came at the last rank. In the second season, the sorghum and mixed substrates came at the first rank followed by maize then wheat came at the last rank. The cap weight followed the same trend of cap diameter in both seasons.

Stipe diameter affected by the different types of grain substrates used for spawning. In the first season, maize spawn gave the highest value followed by sorghum and wheat spawn were ranked second with no significant differences between them. The mix (1:1:1) spawn came in the last place, resulting in less value for stem diameter. The stipe diameter of the second season did not follow the same trend as the first season. Furthermore, the mix spawn (a ratio of 1:1:1) was the superior giving the highest stipe diameter value. Followed by sorghum and maize spawn which came in the second place without any significant differences, and wheat came in last place. Maize grain substrate showed the highest stipe weight at the first season followed by sorghum and wheat then mix substrate. At the second season sorghum substrate achieved the highest stipe weight values followed by the mixed substrate and maize, while wheat substrate showed the lowest values. The mixed substrate showed better fruit bodies quality including cap weight and diameter, stipe weight and diameter. This mixed grain substrate may be achieved better mycelium growth and resulted in better yield and fruit body characteristics. This may be due to the chemical constituents of the mixed grain substrate was better to achieve healthy mycelium growth **(Jarial and Jarial, 2022)**.

Table 1. Effect of some organic substrates and their mixtures on Early yield/bag(gm), Total fresh Yield/bag (gm) to of oyster mushroom

Treatments	Early yield/bag(gm)		Total fresh yield/ bag(gm)		Number of fruit bodies/cluster	
	First season	Second season	First season	Second season	First season	Second season
Sorghum	570.00±25.20 a	427.11±20.55 a	2802.9±9.26 a	2832.3±34.54 a	18.44±2.03 b	21.58±1.51 b
Maize	273.50±11.64 d	391.33±22.17 a	2925.5±195.83 a	2620.4±49.92 b	19.61±3.22 b	28.10±2.42 a
Wheat	357.67±29.16 c	339.00±22.61 b	807.94±67.81 c	2089.2±22.61 c	27.837±3.44 a	19.88±3.03 b
Mix	435.77±35.79 b	412.11±05.12 a	2308.1±75.55 b	2848.2±49.56 a	22.260±2.48 ab	24.25±2.59 ab

Values having the same alphabetical letter(s) did not significantly differ at 0.05 level of significance according to Duncan s multiple range test.

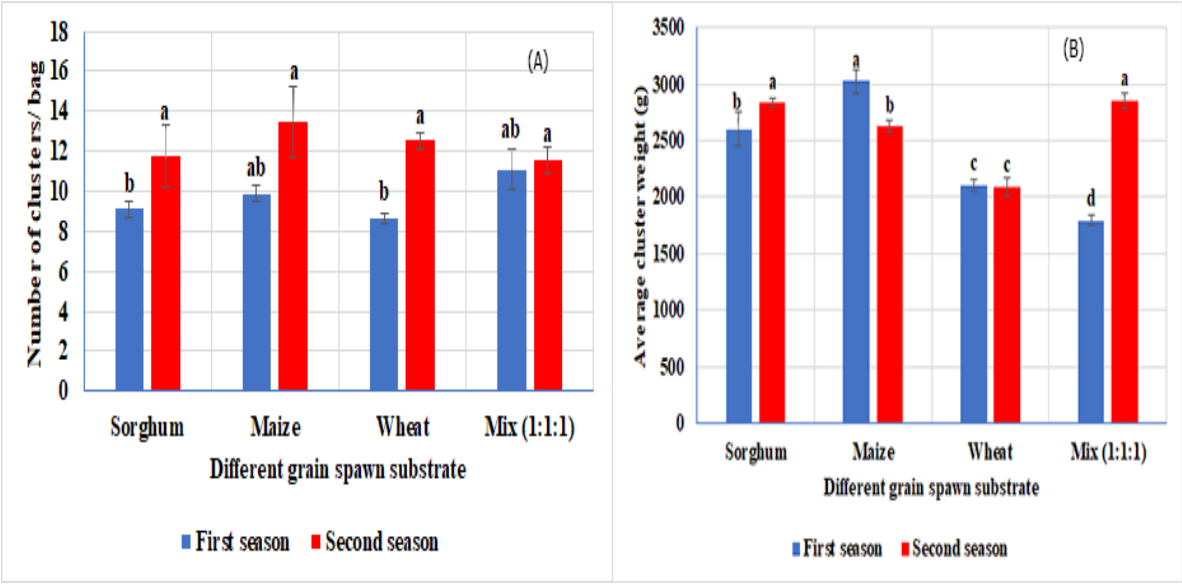

Fig. 1. Effect of different grain spawn substrates on number of clusters per bag (A) and average cluster weight (B) of oyster mushroom (*Pleurotus florida*)

Talpe 2. Effect of different grain substrates and their mixtures used for spawning on cap diameter and stipe diameter of oyster mushroom fruit bodies

Treatments	Cap diameter (cm)		Cap weight (g)		Stipe diameter (cm)		Stipe Weight (g)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Sorghum	33.63±1.21 b	42.29±1.1 a	149.00±4.48 b	229.67±14.10 a	12.34±0.23 b	15.390.01± ab	34.78±2.99 ab	28.44±2.31
Maiz	43.06±2.09 a	36.69±0.4 b	194.56±1.81 a	204.33±04.50 b	15.09±1.53 a	13.97±0.74 b	36.94±4.52 a	24.11±1.10
Wheat	36.50±2.88 b	27.67±1.6 c	155.33±6.84 b	136.50±10.35 c	11.27±0.30 b	10.28±0.41 c	28.17±1.70 bc	18.17±0..47
Mix	24.73±1.73 c	41.54±0.6 a	121.89±6.72 c	236.33±08.55 a	8.74±0.27 c	16.71±0.59 a	23.67±2.23 c	26.00±0.27

Finally, it was found that using sorghum grain substrate was the best as a medium for spawn run, in addition using a mixed grain as a medium showed higher performance. This mixed grain substrate can be applied for oyster mushroom spawn run for higher productivity and yield characteristics

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استخدام بيئة حبوب مختلفة لإنتاج تقاوي عيش الغراب وتعظيم إنتاج فطر عيش الغراب المحاري (*Pleurotus florida*)

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أجريت هذه الدراسة خلال الفترة من 2021- 2022 بمعمل أبحاث عيش الغراب ، التابع لقسم البساتين ، كلية الزراعة – جامعة الزقازيق يعد تحسين جودة الإنتاج أمرًا بالغ الأهمية لنجاح زراعة فطر عيش الغراب، ويعتمد على استخدام الحبوب كبيئة لنمو الجراثيم وإعطاء التقاوي. في هذه الدراسة، تم تطوير انتاج التقاوي باستخدام الحبوب المختلفة. تم تلقيح الحبوب المختلفة بجراثيم عيش الغراب، بما في ذلك الذرة والذرة الرفيعة والقمح وخليط من الذرة: الذرة الرفيعة: تفرخ القمح (1:1:1). بعد التحضين، تمت زراعة التقاوي على بيئة قش الأرز، مما أظهر تأثيرات متباينة على عدد الأيام من الزراعة حتى اول جمع (15-33 يومًا). ومن الجدير بالذكر أن استخدام حبوب الذرة الرفيعة أظهر أعلى إنتاجية في كمية المحصول وحجم الثمار، يليه خليط القمح والذرة الرفيعة في كلا الموسمين. لعب التكوين الغذائي للذرة الرفيعة وخليط 1:1:1 دورًا محوريًا في تحفيز نمو فطر عيش الغراب المحاري، مما يؤدي الي تحسين كمية المحصول والخصائص المورفولوجية للثمار. وبالنظر إلى هذه النتائج والدراسات السابقة، يوصى بشدة باستخدام حبوب الذرة الرفيعة كبيئة وسيطة لنمو جراثيم فطر عيش الغراب. علاوة على ذلك، فإن استخدام تقاوي الذرة العويجة كحامل للجراثيم يمكن أن يعزز جودة التقاوي، ويعزز النمو بشكل أسرع وأكثر قوة. وتهدف هذه الدراسة إلى تعظيم الإنتاجية، والجودة لعيش الغراب المحاري.

المحكمـــان:

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